REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
2008	Journal Article - Science Direct	

4. TITLE AND SUBTITLE

Thermoregulatory Responses to Environmental Toxicants: The Interaction of Thermal Stress and Toxicant Exposure

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

L.R. Leon

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Thermal and Mountain Medicine Division
U.S. Research Institute of Environmental Medicine
Natick, MA 01760-5007

8. PERFORMING ORGANIZATION REPORT NUMBER

MISC07-36

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Same as #7 above.

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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15. SUBJECT TERMS

Toxicology; Hypothermia; Fever; Heat stress; Organophosphates

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Lisa Leon
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(Include area code)* 508-233-4862

Available online at www.sciencedirect.com
ScienceDirect
Toxicology and Applied Pharmacology 233 (2008) 146–161
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Thermoregulatory responses to environmental toxicants: The interaction of thermal stress and toxicant exposure

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Available online 31 January 2008

Abstract

Thermal stress can have a profound impact on the physiological responses that are elicited following environmental toxicant exposure. The efficacy by which toxicants enter the body is directly influenced by thermoregulatory effector responses that are evoked in response to high ambient temperatures. In mammals, the thermoregulatory response to heat stress consists of an increase in skin blood flow and moistening of the skin surface to dissipate core heat to the environment. These physiological responses may exacerbate chemical toxicity due to increased permeability of the skin, which facilitates the cutaneous absorption of many environmental toxicants. The core temperature responses that are elicited in response to high ambient temperatures, toxicant exposure or both can also have a profound impact on the ability of an organism to survive the insult. In small rodents, the thermoregulatory response to thermal stress and many environmental toxicants (such as organophosphate compounds) is often biphasic in nature, consisting initially of a regulated reduction in core temperature (i.e., hypothermia) followed by fever. Hypothermia is an important thermoregulatory survival strategy that is used by small rodents to diminish the effect of severe environmental insults on tissue homeostasis. The protective effect of hypothermia is realized by its effects on chemical toxicity as molecular and cellular processes, such as lipid peroxidation and the formation of reactive oxygen species, are minimized at reduced core temperatures. The beneficial effects of fever are unknown under these conditions. Perspective is provided on the applicability of data obtained in rodent models to the human condition.
Published by Elsevier Inc.

Keywords: Toxicology; Hypothermia; Fever; Heat stress; Organophosphates

Introduction

The interaction of thermal stress and chemical toxicity has been an area of active laboratory investigation for over a century. It has been recognized since the late 1890s from studies conducted in small laboratory species that heat exposure tends to exacerbate chemical absorption and toxicity profiles, whereas cold exposure tends to inhibit these responses. However, a linear relationship between thermal stress and toxicity is not universal for all classes of chemicals and is significantly affected by changes in core temperature (T_c) that are elicited by these stimuli. Thermal stress, chemical exposure or the combination of the two stressors can elicit profound decreases (hypothermia; Gordon and Fogelson, 1993; Malcolm and Alkana, 1983) or increases (hyperthermia or fever; Fine et al., 1997; Gordon, 1997) in T_c — in turn, these T_c changes alter the physiological processes of absorption, distribution, metabolism and excretion, which ultimately determine the systemic toxicity of a chemical.

There are a variety of civilian and military occupations that predispose individuals to the combination of thermal stress and toxicant exposure. For example, the incidence of pesticide or insecticide poisoning increases in frequency during the hot summer months when the application of these chemicals is critical for agricultural success. Similarly, increased frequency of lead poisoning in children has been observed in the summer months (Baetjer, 1959) — is the increase in toxicity in these cases simply a function of more frequent exposure to a chemical or is there an interaction between heat stress and chemical exposure that exacerbates toxicity? Several experimental studies have been designed to specifically address this question and provide data supporting temperature–drug interactions as the cause for increased incidence of poisoning in these cases. Firefighting does not represent a seasonal occupation, but encompasses exposure to thermal extremes in confined spaces

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0041-008X/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.taap.2008.01.012

and during the wearing of protective gear (e.g., fire-retardant clothing, self-contained breathing apparatus) and chemical applications that can be dangerous to the health of the individual (Bruce-Low et al., 2007; Holmer et al., 2006). Racecar drivers are required to perform highly technical operations under conditions of extreme heat (inside car temperatures ≥ 50 °C), wearing of heavy protective clothing, exposure to elevated carbon monoxide levels, and high metabolic rates (Walker et al., 2001) — how do these factors combine to affect the individual's capacity to perform high speed operations in a safe manner?

Similar concerns impact military populations as they are exposed to a multitude of stressors (psychological stress, heat stress, dehydration, exercise) in an unpredictable manner. Protective clothing may be a significant pre-disposing factor to thermal stress due to the weight of the gear and the insulation it provides between the skin and the environment, the latter of which impedes heat exchange. Severe sweating during the wearing of these clothing ensembles may impair fabric integrity, increasing the opportunity for toxicants to be absorbed into the fabric and onto the skin surface or cause such discomfort that personnel remove some or all of the clothing to more effectively perform their duties (Snodgrass, 1992; Wester et al., 1996).

There are a variety of physiological and behavioral adjustments that occur during exposure to thermal stress that can significantly impact the absorption, distribution, metabolism and excretion of a chemical. One of the most significant variables to affect chemical toxicity is temperature — not only the ambient temperature (T_a), which may have a direct effect on the properties of a chemical (e.g., vapor pressure; Chang and Riviere, 1991), but T_c as well. Hypothermia and fever are common thermoregulatory responses observed in laboratory species and humans following heat stress and chemical poisoning (alone or in combination with one another) and although we do not fully understand the mechanisms, experimental data support the hypothesis that hypothermia is protective and may have clinical benefit for the prevention of toxicant injury (Craig et al., 1959a; Gordon, 1997; Malcolm and Alkana 1983). Interestingly, fever is the more common response observed in poisoned humans, but the benefit of this response remains unrecognized (Christoph, 1989; Fine et al., 1997). Provided below is an overview of our current understanding of T_a, T_c and chemical interactions *in vivo* and the effect(s) of these variables on chemical toxicity.

Interaction of core temperature and drug action

> "…the definition of the action of a drug…must be defined as the reaction between the drug and the various parts of the body at a certain temperature" Brunton, 1874

T_c has a direct effect on all biochemical and physiological processes of the body, making it an important variable to consider with respect to the pharmacodynamics of drug toxicity. The impact of T_c on drug action is typically described in terms of the Q_{10} effect which states that for each 10 °C increase (or decrease) in T_c, there is a 2- to 3-fold increase (or decrease) in enzymatic reaction rates. A variety of physiological processes important for drug reactivity, such as receptor binding, lipid peroxidation, and oxidative phosphorylation are temperature-sensitive reactions — thus, T_c is often directly correlated with the magnitude and inversely correlated with the duration of a drug response (Doull, 1972; Klaassan and Eaton, 1991). Of course, the strength of the temperature–drug interaction is dependent on a variety of factors specific to the drug (e.g., drug class), the subject (e.g., species, age, and sex) and/or the experimental design. Fuhrman and Fuhrman (1961) described three types of toxicity curves based on studies conducted in mice and rats over a wide range of environmental temperatures. The type A toxicity curve is depicted as a V- or U-shaped function with minimal toxicity observed at temperatures close to or equivalent to thermoneutrality (T_a associated with minimal metabolic rate; IUPS Thermal Commission, 2001) and increasing toxicity at lower and higher temperatures. The drugs that fall into this category are considered those that interfere with CNS thermoregulatory control mechanisms, such as chlorpromazine, morphine, and salicylates (Fuhrman and Furhman, 1961). The type B toxicity curve is a linear function showing drug toxicity increasing with environmental temperature and is displayed by toxicants such as ethanol and amphetamines (Askew, 1962; Finn et al., 1989). The type C toxicity curve has constant values of toxicity over a wide range of cool temperatures with a sudden linear increase of toxicity at environmental temperatures above the upper limit of thermoneutrality. The validity of this curve has been questioned as drugs originally included in this classification were subsequently shown to have type A or B characteristics (Fuhrman and Fuhrman, 1961).

Four types of experimental designs have been used to determine temperature–drug interactions, including (1) exposure of cell lines, (2) isolated tissue preparations, (3) poikilothermic (cold-blooded) species or (4) homeothermic (warm-blooded) species to a wide range of environmental temperatures and drugs. Cell culture systems are useful for studying toxicological responses due to the ability to strictly control the culture conditions. Although it is important to understand how individual cell populations are differentially affected by drug exposure, it is unlikely that isolated cells accurately reflect the responses that occur in the intact organ where multiple cell populations exist (Perkins et al., 2006). Isolated tissue (*in situ)* preparations are extremely useful as multiple cell populations can be studied simultaneously and drug delivery to the tissue can be strictly controlled. Drug effects have been analyzed using isolated skin (Chang and Riviere, 1991; Wester et al., 1996), muscle (Cavalcante et al., 2005), heart (Gunn, 1914), liver (Lafranconi and Huxtable, 1984), and kidney cells (Lash et al., 2001), which represent the major organs responsible for drug absorption, distribution, metabolism and excretion in the whole body. The use of *in vitro* and *in situ* preparations overcomes several of the experimental limitations of *in vivo* studies, as the concentration of the drug attained at the cellular or tissue level as well as the duration during which the concentration is maintained above a critical threshold can be strictly controlled. Thus, inter-subject (animal or human) differences in drug absorption and distribution are effectively eliminated and no longer a source of experimental variability. Additionally, tissue studies permit direct assessment of the role of a specific organ in drug

pharmacodynamics, which is often difficult to determine in the whole animal. The drawback of *in vitro* and *in situ* studies is that they do not accurately represent whole body exposures in which several physiological systems (thermoregulatory, cardiovascular, nervous, endocrine) influence whole body drug toxicity. To overcome this limitation, laboratory test species are used to examine drug toxicity following whole body exposure.

Poikilotherms, such as frogs and fish, have traditionally been used in chemical toxicity studies due to their ability to withstand a wide range (~35 °C) of T_c fluctuations with little or no adverse effects. Due to poikilotherms having a lack of internal thermoregulatory control (i.e., T_c closely matches T_a), the effect of a range of temperatures on drug toxicity can be directly assessed in these species. Of course, the caveat to these studies is the difficulty in extrapolating research findings to homeotherms, such as man, whose biochemical and thermoregulatory systems are not as readily affected by changes in T_a. Thus, many studies have been conducted in small homeotherms, such as mice and rats, and directly compared to findings produced in poikilotherms. An example of this type of comparative study is provided by Jacobj (1890) who showed that the active alkaloid colchicine is more toxic in mice than frogs, suggesting that the warmer T_c of mammals may enhance drug toxicity. Ideally, a combination of all 4 experimental designs is beneficial for studies of drug toxicity, although this approach may not be practical in all laboratory settings.

Typically, Q_{10} effects on drug toxicity have not been a major consideration of studies conducted in homeotherms due to their ability to maintain a stable T_c over a wide range of environmental conditions. Rather, in homeothermic studies it is generally assumed that T_c is not affected by T_a, drug treatment or the combination of the two stressors — however, this is an invalid assumption as T_c is rarely measured in rodent studies, and homeothermic T_c can fluctuate quite dramatically, ranging from ~20 °C to ~43 °C in response to a variety of environmental stimuli (Buchanan et al., 1991; Gavrilova et al., 1999; Ibuka and Fukumura, 1997; Klein et al., 1992; Leon et al., 2005). An overview of the mechanisms responsible for T_c changes in homeotherms is provided below.

The thermoregulatory control system of homeotherms

The thermoregulatory control system of homeotherms is compromised of a variety of neurophysiological mechanisms that function to maintain core temperature (T_c) at a homeostatic level. Heat transfer mechanisms are elicited in response to changes in body heat storage, the latter of which is dependent on metabolic rate, work and the four avenues of heat exchange, as described in the following equation (IUPS Thermal Commission, 2001):

$$S = M - (W) - (E) - (C) - (K) - (R)$$

where S is heat storage in the body, M is metabolic rate, W is work, and E, C, K and R are evaporative, convective, conductive and radiant heat transfer, respectively (i.e., the four primary modes of heat transfer between the environment and the body). It is important to note that the impact that the four avenues of heat exchange have on total body heat storage is dependent on a variety of organismal (e.g., age, sex, adiposity), environmental (e.g., humidity, wind velocity), and occupational (e.g., protective clothing) variables. Under conditions in which heat production exceeds heat loss, such as during exercise or heat exposure, positive heat storage occurs and T_c increases. Conversely, when heat loss exceeds heat production, such as during prolonged cold exposure, negative heat storage occurs and T_c decreases.

The neurophysiological mechanisms that function to maintain T_c at a homeostatic level have been extensively studied and shown to reside within the preoptic area of the anterior hypothalamus (POAH, also referred to as the thermoregulatory integration center; Hammel, 1968; Heath et al., 1972; Simon, 1981). The POAH and other central nervous system (CNS) sites are regions of the brain that respond to displacements in T_c by eliciting corrective autonomic and behavioral effector mechanisms to alter heat storage and return T_c to its baseline level. A diagrammatic representation of this negative feedback loop of T_c homeostasis is shown in Fig. 1 and has been described in detail elsewhere (Gordon, 2005). In an effort to describe the changes occurring in the POAH that drive thermoregulatory responses, the concept of a "thermal setpoint" has been developed and, although theoretical in nature, provides a framework upon which to explain differences between regulated and unregulated changes in T_c. The thermal setpoint is generally described as being analogous to a thermostat that controls a mechanical heating device — under homeostatic (baseline) conditions, temperature oscillates around a setpoint value (T_{set}; ~37 °C, although this value differs between species), such that $T_c \approx T_{set}$ (Fig. 2). When an environmental stimulus perturbs T_c so that it is no longer equivalent to T_{set}, the organism becomes

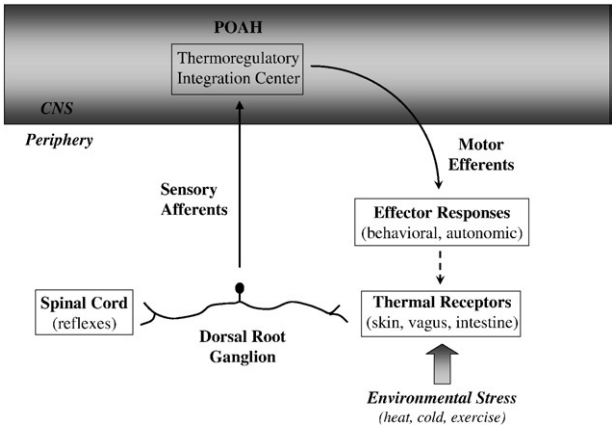

Fig. 1. Diagrammatic representation of the negative feedback pathway regulating core temperature in homeotherms. Thermal receptors sense changes in core temperature following exposure to an environmental stressor, such as heat or cold. The thermal receptors send an afferent signal through the dorsal root ganglion either directly to the spinal cord for reflexive actions or to the preoptic area of the anterior hypothalamus (POAH). The POAH acts as a thermoregulatory integration center, comparing sensory temperature information to setpoint temperature and responds with corrective behavioral and autonomic thermoeffector responses to return core temperature to its baseline level. ——▶ indicates stimulatory pathway, - - - - - - - - ▶ indicates inhibitor pathway.

either hyperthermic ($T_c > T_{set}$; Fig. 2A) or hypothermic ($T_c < T_{set}$; Fig. 2C). When heat storage is altered in the absence of a change in setpoint, these perturbations represent unregulated changes in T_c — under these conditions, corrective effector responses are elicited to alter heat storage and return T_c to its original baseline level. This has been demonstrated experimentally in rodents implanted with thermodes in the POAH — following localized warming or cooling of this CNS site, which simulates changes in heat storage in the absence of a setpoint change. Corrective effector mechanisms such as changes in vasomotor tone and metabolic rate are elicited to return T_c to its baseline level (Heath et al., 1972).

Regulated changes in T_c occur in response to a change in the thermal setpoint and represent adaptive responses used by the organism to survive and mitigate potential adverse effects of an environmental insult. Regulated hyperthermia, or fever, is a common response to infection, inflammation and trauma and occurs in response to an increase in the thermal setpoint — to match T_c to an elevated setpoint level, increases in heat gain (shivering, drinking of warm fluids) and decreases in heat loss (vasoconstriction, huddling) are elicited (Fig. 2B). Liebermeister (1887) was the first to accurately define fever as a regulated elevation in the thermal setpoint when he observed a return of febrile individuals to a previous level of T_c following experimental warming or cooling. Fever is regarded as an adaptive response as it enhances several immune responses, such as interferon activity and leukocyte function, to limit the sequelae associated with infectious or inflammatory conditions (Heron and Berg, 1978; Johansen et al., 1983). Regulated hypothermia (also referred to as anapyrexia; IUPS Thermal Commission, 2001) represents the condition in which T_c is lowered in response to a decrease in the thermal setpoint (Fig. 2D). A reduction in setpoint evokes a variety of effector mechanisms that promote heat loss (vasodilation, splaying to increase surface area, seeking of cool environments), diminish heat production (reduction in metabolism) and lower T_c to match the new decreased setpoint level. Once fever or anapyrexia has been attained, $T_c \approx T_{set}$ at the newly elevated or decreased setpoint level, respectively. Regulated hypothermia imparts a survival advantage by decreasing metabolic demands under conditions of severe energy depletion, tissue injury or infection and is used by several species to survive environmental insults, such as food restriction (Buchanan et al., 1991; Graf et al., 1989), hypoglycemia (Buchanan et al., 1991), hypoxia (Malvin and Wood, 1992), hemorrhage (Brown et al., 2005), dehydration (Ibuka and Fukumura, 1997), infection (Klein et al., 1992; Leon et al., 1998), heat stroke (Leon et al., 2005; Romanovsky and Blatteis, 1996) and drug toxicity (Gearhart et al., 1993; Gordon, 1997). The survival value of regulated hypothermia and fever is supported by studies showing that the prevention of these responses increases morbidity and mortality to infection and other environmental stressors (Buchanan et al., 1991; Leon et al., 2005; Malvin and Wood, 1992). In many instances, these T_c changes represent regulated changes that impart a survival advantage by limiting the temperature-dependent processes of absorption (GI tract, skin, lungs), distribution (circulatory adjustments), metabolism (liver, kidney) and excretion (kidney) of a drug, which are ultimately responsible for the concentration attained at the tissue level. Provided below is a brief description of the physiological responses that are elicited in response to heat exposure and how these changes may alter drug exposure and toxicity in homeotherms.

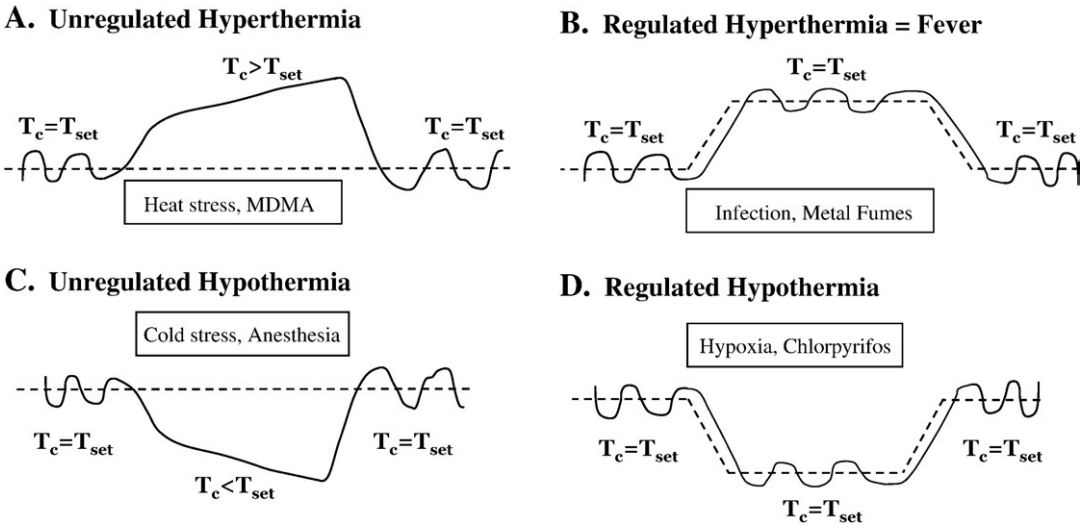

Fig. 2. Diagrammatic representation of the theoretical concept of core temperature (T_c) changes mediated by a change in the thermal setpoint (T_{set}). Note that under all conditions, at baseline (i.e., normothermia) T_c oscillates around T_{set}. (A) *Unregulated Hyperthermia*. Following an external perturbation, such as heat stress or MDMA (ecstasy) ingestion, T_c is elevated in the absence of a setpoint change. Although thermoeffector mechanisms are elicited to resist the increase in T_c, they are ineffective in preventing the hyperthermia until the external perturbation is removed. (B) *Regulated Hyperthermia or Fever*. In response to infection or a toxicant, an increase in T_{set} elicits a variety of thermoeffector responses that cause heat production to exceed heat loss and T_c is set to an elevated level. (C) *Unregulated Hypothermia*. Following an external perturbation (e.g., cold stress, anesthesia), T_c is decreased in the absence of a setpoint change as thermoeffector responses aimed at increasing heat gain/production cannot counteract the increase in heat loss. Once the external perturbation is removed, thermoeffector mechanisms (vasoconstriction, shivering) are effective at returning T_c to $T_{set.}$ (D) *Regulated Hypothermia or Anapyrexia*. In response to hypoxia or a toxicant, a decrease in T_{set} elicits a variety of thermoeffector responses (e.g., vasodilation, splaying to increase surface area) that cause heat loss to exceed heat gain/production, driving T_c to a decreased level.

Physiological responses to heat stress

Physiological adjustments to heat exposure consist of a variety of autonomic and behavioral responses that are aimed at maintaining T_c at a non-lethal level. The primary autonomic responses aiding in heat dissipation include vasodilation (increased blood flow from the core organs to the skin surface), sweating and increased ventilation. Increased skin blood flow facilitates dry heat transfer between the core and the environment. The maintenance of high skin blood flow can induce significant cardiovascular strain as a high heart rate is required to maintain cardiac output while a decrease in splanchnic blood flow induces GI ischemia and increases vascular permeability. The latter response facilitates the leakage of endogenous bacteria from the GI tract into the systemic circulation, resulting in endotoxemia — prolonged ischemia to the GI tract and other organs (kidney, spleen, liver) also contributes to the development of tissue injury and other long-term adverse consequences of heat stroke.

In humans, increased skin blood flow occurs concomitantly with activation of eccrine sweat glands, which secrete fluid onto the skin surface for evaporative cooling. Fluid losses from excessive sweating may be as high as 2 L/h under conditions of prolonged heat exposure, which if not adequately replenished, can induce significant dehydration. Adolph and associates (1947) reported ~2–8% body weight reductions in troops exercising in the heat whereas animal studies have shown reductions as high as ~13% following ~5 h heat exposure (Leon et al., 2005). The respiratory surfaces represent an important avenue for evaporative and dry heat loss. Panting is an effective method of evaporative heat dissipation in large animals such as birds, dog, sheep and rabbits — although panting would appear to be a costly mechanism of cooling, in many species it is performed at a resonant ventilatory frequency that minimizes energy expenditure (Calder and Schmidt-Nielsen, 1966; Crawford, 1962; Hammel et al., 1958). Non-panting homeotherms, such as humans and rodents show an increase in breathing frequency and minute volume during heat exposure, which facilitates evaporative cooling from the respiratory surfaces, but is not representative of panting *per se.* In rodents, avoidance behavior (increased locomotor activity) has been noted during prolonged heat exposure, which stimulates metabolism and an increase in T_c and ventilation rate. In humans, an increase in the ventilation rate occurs as exercise or high metabolic activities are often performed during heat exposure.

Behavioral mechanisms of T_c homeostasis vary slightly between species, but will be used preferentially, when available and effective, over autonomic mechanisms as they require minimal energy expenditure to sustain. Examples of behavioral thermoregulatory mechanisms in humans include the seeking of shade or cool microclimates, the discontinuation of exercise or high metabolic activities, the consumption of cool fluids, and removal of clothing. Unfortunately, many individuals remove protective clothing even when exposed to occupational hazards that the clothing is designed to protect against (e.g., pesticide exposure) due to the impermeable nature of the clothing, which inhibits heat transfer between the skin and the environment. In animals, evaporative cooling is achieved through a combination of autonomic and behavioral mechanisms as the secretion of saliva is stimulated autonomically, but requires behavioral spreading onto the fur surface to facilitate evaporation. Unfortunately, many experimental animal studies limit opportunities for behavioral spreading of saliva by the use of restraint and/or anesthesia in the experimental design. Additional animal heat dissipation behaviors include the seeking of cool environments (many desert species are nocturnal and primarily active during the cool night) and changes in piloerection (stimulation of the hair follicles to alter the barrier of air between the skin and the environment).

During prolonged heat exposure, autonomic mechanisms of thermoregulatory control, such as sweating may become exhausted, resulting in dramatic increases in T_c with values as high as ~47 °C reported in patients (Bouchama et al., 1991; 1993; Chang, 1993; Hammami et al., 1997; Hashim et al., 1997; Lu et al., 2004; Sonna et al., 2004). Hypothermia (~7 °C below baseline) and fever (~1–2 °C above baseline) are long-term heat stroke recovery responses that are less well-recognized, but are thought to be a consequence of the systemic inflammatory response (SIRS) that occurs in response to endotoxin leakage from the ischemic GI tract (Attia et al., 1983; Austin and Berry, 1956; Leon et al., 2005; Malamud et al., 1946; Romanovsky and Blatteis, 1996; Wilkinson et al., 1988; Wright, 1976). These T_c responses have also been observed following toxicant exposure. As discussed below, a potential impact of thermal stress on chemical toxicity has been recognized for decades, although we still do not have a clear understanding of the pathophysiological mechanisms that mediate the adverse consequences of this interaction in the whole animal.

Effect of environmental temperatures on drug toxicity

Heat exposure

The primary routes by which toxicants enter the body include the respiratory surfaces, the GI tract and the skin (Casarett and Doull, 1975); as described above, the function of all of these organ systems is altered during heat exposure. Inhalational exposures may be enhanced during heat stress due to increases in ventilation rate and tidal volume. In rats, a 10 °C elevation in environmental temperature caused a 25% increase in toxicity to inhaled nitrogen dioxide, which was likely a consequence of inhalation of a larger volume at the high temperature (Gray et al., 1954). In exercising dogs the retained dose of sarin was found to be directly proportional to inhalation volume (McLemore and Craig, 1959). Sarin toxicity is increased at high temperatures, which may be directly related to its actions on peripheral vasomotor tone (Craig et al., 1959b; Stephenson and Kolka, 1990). In Rhesus monkeys a ~60% increase in sarin-induced mortality occurred at 38 °C, which correlated with a reduction in skin temperature and a significant increase in T_c (as high as 42.4 °C in some animals; Craig et al., 1959a). These data suggest that the vasoconstrictive effects of sarin inhibited the ability of these monkeys to dissipate core heat to the

environment during heat exposure such that they succumbed to heat stroke, rather than the direct effects of sarin toxicity.

Most studies have examined temperature–drug interactions while neglecting to measure T_c of the experimental subject. Keplinger et al. (1959) examined toxicity to 58 compounds in rats maintained at 8, 26 or 36 °C for ~72 h and neglected to record T_c. The rats exposed to 36 °C succumbed at 2- to 17-fold lower doses of each compound compared to rats housed at the lower temperatures (Keplinger et al., 1959). Although it was not universal for all compounds tested, the onset of drug action was more rapid and the survival period shorter at the highest temperature (Keplinger et al., 1959). Unfortunately, because T_c was not measured in this study, it is not possible to determine if the environmental temperature increased rat sensitivity to the drug or if the drug increased thermal sensitivity, causing the animals to collapse from heat stroke.

The widespread use of organophosphate compounds (OPs; insecticides, pesticides, nerve gases) throughout the world has stimulated research into understanding the environmental conditions that influence absorption and consequent toxicity of these compounds. The primary mechanism of OP toxicity is the inhibition of acetylcholinesterase (AChE) enzyme activity. The function of AChE is to degrade acetylcholine (ACh) concentrations at CNS and peripheral nerve terminals and limit the time course of ACh actions. ACh binds to nicotinic and muscarinic receptors in the CNS and peripheral nervous system and alters a variety of effector responses that affect T_c homeostasis, including skin blood flow, heart rate, respiration, and sweat (or salivary) gland secretion. Following OP poisoning, the inhibition of AChE functioning and prolongation of ACh actions at postganglionic nerve terminals induces a "cholinergic crisis" characterized by excess salivation, diarrhea, muscle tremor, hypothermia, fever, and other symptoms (Cupp et al., 1975; Hassan et al., 1981; Klemmer et al., 1978). The level of OP toxicity is determined by AChE inhibition levels in the plasma or serum, with the assumption that these levels also reflect changes occurring in the brain. In a few animal studies, behavioral and autonomic dysfunction has been shown to occur when AChE inhibition exceeds ~50% (Glow and Rose, 1965; Gordon and Fogelson, 1993; Kozar et al., 1976). However both human and experimental animal studies have shown decrements in physiological and mental performance occurring over a wide range of AChE inhibition levels (22–95%). On the other hand, the level of serum or erythrocyte AChE inhibition (typically used as an indirect measure of the level of tissue AChE inhibition) may be detectable in the absence of toxicity symptoms or may persist after recovery from symptoms (Carr and Chamgers, 1991; Namba et al., 1971; Wenger et al., 1993), indicating that this is an inaccurate measure of toxicity that cannot be used for the establishment of exposure limits (Clement, 1991; Gordon and Fogelson, 1993; Maickel et al., 1991; Namba et al., 1971). Despite these caveats, in humans atropine (a competitive inhibitor of ACh actions) is the treatment for OP poisoning with the re-establishment of normal AChE activity correlating with clinical improvement (Hassan et al., 1981).

OPs are generally considered irreversible inhibitors of AChE due to the slow dissociation rate of their binding to the active site of the enzyme. However, there are reversible agents that have been used for the treatment of myasthenia gravis and as prophylactic agents against chemical warfare agents (Hood, 1990; Keeler et al., 1991). Myasthenia gravis is a chronic autoimmune neuromuscular disease characterized by varying degrees of weakness of the skeletal muscles of the body. In this disease, antibodies block, alter, or destroy the receptors for ACh at the neuromuscular junction and prevent muscle contraction — the use of pyridostigmine bromide (PYR), a carbamate compound that binds reversibly to AChE, increases ACh concentrations at the neuromuscular junction and improves muscular function in these patients. Due to its reversible nature, PYR has also been fielded by the Armed Forces of the U.S. and other countries as a pretreatment against potential nerve gas poisoning. The use of PYR by military personnel is designed to improve the effectiveness of post-exposure atropine treatment and protect against the debilitating effects of nerve agents, such as sarin or soman, that cause loss of muscle control and death from respiratory failure. Self-administration of PYR in myasthenia gravis patients or military personnel is distinct from that occurring during accidental poisoning, in that exposure is typically chronic, as it is administered over several years in patients or for several days during engagement in warfare maneuvers that carry the threat of a nerve agent attack. Adverse responses to chronic PYR treatment have been reported in patients, including increased salivation, increased lacrimation, nausea, vomiting, and diarrhea (Keeler et al., 1991). Although Soldiers of the Gulf War have complained of similar symptoms, a retrospective study of 41,650 Soldiers showed that less than 0.1% had effects sufficient to warrant discontinued use of the drug (Keeler et al., 1991). Similarly, an analysis of risk factors for unexplained illness in a population-based sample of Gulf War Veterans suggests that illness is most highly associated with combat conditions and heat stress, whereas interactions of PYR, insecticides, repellents, and stress were not significant (Spencer et al., 2001).

The two main effects of AChE inhibition that need to be considered with respect to risk assessment of human populations performing work in hot environments are the alterations that may occur in sweat rates and skin blood flow. In myasthenia gravis patients, warm temperatures may induce clinical symptoms due to direct effects on the release of ACh, endplate sensitivity, and AChE activity (Rutkove, 2001). Whole-body cooling of these patients has shown promise in decreasing the symptoms of weakness and fatigue associated with this disease (Mermier et al., 2006). In populations performing work in hot environments, increased sweat production induced by PYR treatment or OP poisoning is expected to facilitate evaporative cooling and improve heat tolerance through the maintenance of T_c at non-harmful levels. In human volunteers, exposure to acute or chronic PYR treatment was shown to induce increases in sweating rates and evaporative water loss during exercise in either a temperature (29 °C) or hot (42 °C) environment, with little or no effects on T_c (Epstein et al., 1990; Stephenson and Kolka, 1990; Wenger et al., 1993; Wenger and Latzka, 1992). Changes in skin blood flow are also important for T_c

homeostasis, but there is controversy regarding the effect of PYR and the actions of ACh on skin blood flow responses during exercise-heat stress. Although increases in skin blood flow have been shown to occur in response to ACh in humans or isolated mammalian blood vessels (Carmichael and Fraser, 1933; Furchgott and Aawadzki, 1980), atropine administration inhibited vasodilation during whole body heating in one study (Roddie et al., 1957) and enhanced vasodilation and forearm blood flow in another (Kolka and Stephenson, 1987). Similar results have been described for PYR treatment in human volunteers in which little or no effect has been shown on skin temperature and T_c, despite significant reductions in AChE activities (Stephenson and Kolka, 1990). Interestingly, skin blood flow may not be similarly affected at all body sites, as chronic PYR treatment caused 0.7 °C reduction in chest skin temperature, but left calf and upper arm skin blood flow was unaffected (Wenger et al., 1993). Presumably, the differences in PYR responsiveness between these studies is related to differences in treatment strategies (acute vs. chronic), environmental temperature (29 °C vs. 42 °C) or other unknown factors. Overall, clinical evaluations of Gulf War veterans and experimental studies conducted on human volunteers have shown that PYR treatment has little effect on physiological or cognitive performance during exercise-heat stress conditions.

The skin is the largest organ of the body and represents a primary route of exposure for environmental toxicants. Sophisticated *in vitro* flow-diffusion systems have been developed to examine the impact of environmental and physiological factors on percutaneous absorption rates of OP compounds. In these *in vitro* preparations, a layer of skin is placed into a chamber in which the temperature, and humidity of the air or the flow rate of the perfusate bathing the underside of the skin can be manipulated in isolation or in combination with one another. Using this setup, the outermost skin layer of the epidermis, known as the stratum corneum is exposed to air while the dermis is exposed to fluid (i.e., perfusate) — by manipulating air and perfusate conditions, it is possible to determine the impact of environmental and simulated physiological conditions on transdermal flux rates of various chemicals. In studies by Chang and Riviere (1991) and Chang et al. (1994), elevation of air temperature to 42 °C moderately increased the transdermal flux rate of the OP pesticide parathion across porcine skin layers, but the effect was not as strong as when both air and perfusate temperature were elevated to 42 °C. These conditions were designed to test the effect of an increase in environmental temperature and the reflexive adjustments in skin blood flow and skin temperature that occur in response to heat exposure on OP absorption rates. High humidity levels also enhanced parathion penetration either due to an increase in the rate of hydration of the stratum corneum and/or change in the vapor pressure of the chemical (Chang and Riviere, 1991). Several hypotheses were suggested to explain the effects of high humidity and temperature on transdermal flux rates, including an increase in the thermodynamic activity of parathion in the lipid bilayers, an increase in stratum corneum lipid fluidity, an increase in corneal hydration or alteration of epidermal function in another, as yet undefined, manner (Chang and Riviere, 1991). To determine how simulated physiological adjustments to heat exposure may affect transdermal absorption rates, the effect of an increased perfusate flow rate (simulates increased skin blood flow) and protein concentration (simulates dehydration) were examined. Under both of these conditions, which were tested in isolation from one another, parathion absorption rates were significantly enhanced, which was thought to be due to more rapid washout of the penetrated compound from the perfusate and more effective binding to perfusate proteins, respectively (Chang and Riviere, 1991). Clearly, the data from these *in vitro* studies indicate that environmental and physiological variables are not independent with respect to their influence on the transdermal absorption of toxicants.

Prior to the development of *in vitro* flow-diffusion systems, the majority of data on the physiological effects of OP exposure were obtained from persons experiencing occupational or suicidal exposures. Under these conditions, persons are examined following clinical presentation with little information regarding exposure duration, quantity of toxicant ingested or absorbed, ambient temperature, humidity, etc. To overcome these limitations and determine mechanisms of toxicity under controlled conditions, studies were performed on human volunteers dermally exposed to OP compounds under a variety of environmental conditions. The strength of *in vivo* studies lies in the ability to expand beyond an analysis of simple diffusion effects on transdermal flux rates, which is the primary measure obtainable using *in vitro* systems, to an examination of several physiological systems that affect whole body toxicity. In a study by Funckes et al. (1963), human volunteers were dermally exposed to 2% parathion at 15, 21, 28 and 41 °C with changes in AChE activity and urinary excretion rates of paranitrophenol (a metabolite of parathion) used to determine the physiologic effect and magnitude of absorption, respectively. Urinary excretion rates of paranitrophenol increased linearly from low to high temperatures, which would not be unexpected if reflexive decreases in renal blood flow and glomerular filtration rates decreased total urine volume and caused greater retention of parathion in the tissues during heat exposure (Funckes et al., 1963). Unfortunately, urine volume was not measured in this study. Furthermore, changes in AChE activities and clinical symptoms of toxicity were undetectable in the subjects, suggesting that increases in parathion absorption did not translate to changes in physiological actions at the nerve terminals; however, the subjects reported increased sweating at the site of exposure for several days following decontamination, indicating a delayed response that is indicative of a mild cholinergic crisis. Similar effects of elevated temperatures on the dermal absorption of VX nerve gas were reported in human volunteers experiencing 3-hour exposures to low (−18 °C and 18 °C) and high (46 °C) temperatures — the rate of maximal dermal penetration and AChE inhibition was directly correlated with increased skin blood flow and skin temperature at 46 °C (Craig et al., 1977). However, lower doses were much more effective following exposure to the cheek versus the forearm due to differences in skin blood flow and permeability of the stratum corneum between these skin layers (Craig et al., 1977). Thus, findings from *in vivo* and *in vitro* studies suggest skin blood flow changes, which may differ across body sites, affect transdermal

absorption rates via several potential mechanisms, including increases in skin surface temperature, hydration state of the stratum corneum, solubility of the chemical in the blood, and the volume of dermal tissue perfused (Riviere and Williams, 1992). It is also important to recognize that these effects can be mediated in individuals whose skin is not directly exposed to a toxicant in the air, such as during the wearing of protective clothing. These clothing ensembles, whose impermeable nature inhibits heat transfer between the skin and the environment, can induce significant increases in sweating rates above that observed in individuals whose skin is directly exposed to the air. Although this clothing is designed to protect individuals from chemical exposure, Snodgrass (1992) showed that chemicals on the clothing surface can be absorbed from the fabric into the skin and the systemic circulation; furthermore, the wetting of fabric, such as occurs with copious sweating, can solubilize chemicals in the fabric and facilitate skin absorption and toxicity (Wester et al., 1996). Thus, while the removal of protective clothing during operations at environmental extremes represents a primary risk factor for exposure, there are indications that reflexive adjustments to heat exposure that wet the inner surface of the clothing may compromise the protective barrier and increase exposure as well.

The effect of elevated temperatures on chemical absorption/toxicity profiles is not specific to dermal exposures or to species that use sweating as their primary mechanism of evaporative cooling. Many studies have used intraperitoneal (i.p.) and intravenous (i.v.) injections to normalize the total drug dose between animals and avoid inter-subject variability in skin or GI absorption rates. In studies by Baetjer et al. (1960) and Baetjer and Smith (1956), the effects of environmental temperature on lead and parathion toxicity were determined by housing the animals at an environmental temperature of 15, 22 or 35 °C for 3 to 5 days prior to i.p. or i.v. injection of the chemicals. While the mortality rates were higher at 15 °C and 35 °C compared to 22 °C, the time course of death differed significantly between the high and low temperatures, indicating different mechanisms of toxicity. At 35 °C, the latent period to the time-of-death was shorter, with the greatest mortality observed during the first 120 h after injection (Baetjer et al., 1960; Baetjer and Smith, 1956). In contrast, mice housed at 15 °C showed a high rate of mortality during the latter part of the recovery period. Thus, the average survival time increased with a decrease in environmental temperature, although the cumulative mortality rate was virtually identical between groups (Baetjer et al., 1960; Baetjer and Smith, 1956). Unfortunately, the mechanisms responsible for these differences in the rate of lethality were not delineated in this study. Interestingly, the detrimental effect of heat was enhanced when exposure to the elevated temperature was provided *following* the lead or parathion injection (without pre-exposure), suggesting that physiological mechanisms of heat acclimatization may provide protection against toxicity.

The primary physiological adaptations to heat acclimatization include an earlier onset and higher rate of skin blood flow, more rapid evaporative cooling response, with earlier onset and more dilute concentration of sweat, and a decrease in basal metabolic rate (BMR). Heat acclimatization studies performed in mice show decreased concentrations of lead in the urine and feces, which is reflected by lead retention in the soft tissues of the body (Baetjer et al., 1960; Baetjer and Horiguchi, 1964). The duration of heat exposure impacts urinary excretion rates, with glomerular filtration rates decreased ~67% in rats following 3 weeks acclimatization to 35 °C (Chayoth et al., 1984). Experimentally-induced dehydration (~12% body weight), either through consumption of a high salt solution (saline) or water restriction techniques (in the absence of heat exposure) also increased mortality rates and kidney concentrations of lead in mice and rats (Baetjer and Horiguchi, 1964; Baetjer et al., 1960); surprisingly, in this study a decrease in fecal lead concentration in dehydrated animals was a more significant factor contributing to lead retention in the tissues than a reduction in urine volume (Baetjer and Horiguchi, 1964; Baetjer et al., 1960). Morphological changes to heat acclimatization have also been noted in rodents with the weight of the liver, kidney, lung, brain, heart, spleen, adrenal glands, thyroid, pituitary and testes significantly decreased following 10 weeks acclimatization to 35 °C compared to the weights observed following acclimatization to 22 °C (Ray et al., 1968). The impact of decreased weight on organ function and the pharmacokinetics of toxicants is unknown. Taken together, these studies indicate that high environmental temperature and dehydration, in combination or as separate stressors can have a significant impact on chemical toxicity, although additional studies are warranted to determine the specific mechanisms responsible for these effects. And it is important to note that the described effects are not specific to lead or parathion, as heat exposure has been shown to increase toxicity to several environmental toxicants including carbon monoxide (Walker et al., 2001), heavy metals (nickel, cadmium, lead; Nomiyama et al., 1980), CNS depressants and stimulants (pentobarbital; Keplinger et al., 1959), and organic solvents (ethanol, sulfolane; Gordon et al., 1985; Nomiyama et al., 1980). Interestingly, despite the large body of literature implicating high environmental temperatures (and its impact on physiological functioning) with alterations in chemical toxicity, OSHA guidelines for permissible exposure limits (PELs) do not take this variable into consideration!

Cold exposure

The majority of toxicity studies have been conducted at elevated environmental temperatures to determine the mechanisms responsible for the high incidence of poisoning that occurs during the hot summer months. However, cold exposure can alter drug toxicity profiles and may be an unrecognized, confounding variable in many studies conducted in laboratory rodents. Several studies have shown beneficial effects of acute and chronic cold exposures on chemical toxicity, which typically correlated with the development of hypothermia (discussed in more detail below — see hypothermia section). It is expected that under condition of low T_a, the reduction in metabolic rate that accompanies hypothermia development is beneficial as it inhibits the production of harmful reactive oxygen species (ROS) that cause tissue damage. Just as heat exposure may enhance the retention of drugs in the soft tissues of the body due

to decreased urinary output, Burgat-Sacaze et al. (1982) suggested cold-induced diuresis as a protective mechanism against methylmercury-induced nephrotoxicity in rats, as a lower percentage of the compound is retained in the soft tissues of the body with high urine outputs. Forced diuresis has been shown to significantly reduce the body burden of pesticides, such as pentachlorophenol (PCP; Young and Haley, 1978). Unfortunately, the majority of toxicity studies conducted on small rodents may have inadvertently induced cold acclimatization effects by housing the animals at inappropriately cool temperatures. The T_a at which small rodents are housed can have a large impact on energy expenditure and T_c homeostasis as heat transfer between the body and the environment is largely dependent on the surface area to body mass ratio (SA:M_b); that is, as body mass decreases, SA:M_b increases. As a consequence of this relationship, the transfer of heat between the body and the environment is significantly greater in small rodents, such as mice and rats than in larger homeotherms, such as man. Ideally, small rodents should be housed at a T_a that requires minimal energy expenditure to ensure normal growth rates and physiological functioning. The thermoneutral zone, or TNZ, is defined as the T_a range at which temperature regulation is achieved without regulatory changes in metabolic heat production (IUPS Thermal Commission, 2001). The lower critical T_a threshold for metabolic stimulation ranges from ~26–30 °C in rodents and unfortunately, the majority of toxicity studies in these species have been conducted below this T_a range (typically 20–22 °C). Small rodents are largely dependent on metabolic regulation for thermal homeostasis (Phillips and Heath, 1995) so prolonged stimulation of metabolic rate during housing under cool ambient conditions can alter normal food and water consumption, reproductive fecundity, and general activity profiles and these changes will alter drug pharmacodynamics. Unfortunately, cold acclimatization effects were likely present in the majority of toxicology studies, with little or no consideration of the effect of alterations in growth, metabolism and other physiological functions on observed responses.

Shivering and nonshivering thermogenesis (NST) represent the predominant mechanisms of heat production and T_c maintenance in small rodents exposed to cool environments. During prolonged cold exposure, NST provides the primary heat source as it is more energetically efficient than shivering. NST occurs in the brown adipose tissue (BAT) of small rodents and newborns and is an important source of heat during cold acclimatization (Ricquier et al., 1979), arousal from hibernation or torpor (Lyman, 1990), production of fever (Dascombe et al., 1989), recovery from anesthesia-induced reductions in T_c (Shimuzu and Saito, 1991), and diet-induced thermogenesis (Himms-Hagen et al., 1981). BAT is a densely vascularized and mitochondrial rich tissue (attributes that are responsible for its brown appearance) that is located at distinct anatomical sites, including the interscapular, pericardial, perirenal, and intercostal regions of the body (Cannon and Nedergaard, 2004). BAT thermogenesis is stimulated by hypothalamic neurons that release norepinephrine (NE) onto α- and β-adrenergic receptors located on BAT cell membranes, which activates an enzymatic cascade that breakdowns triglyceride stores to glycerol and free fatty acids (FFA). Increased FFA concentrations stimulate synthesis of a 32 kDa protein unique to BAT, termed uncoupling protein-1 (UCP-1), whose function is to uncouple oxidative phosphorylation from ATP synthesis and produce heat. The protective function of UCPs in reducing ROS production suggests that the antioxidant action of this protein may be a mechanism of protection from toxicity in cold-acclimatized animals (Li et al., 2001). UCP synthesis is also stimulated by T_3 levels that are increased following activation of thyroxine 5′-deiodinase by NE. Heavy metals, such as cadmium and zinc have been shown to impair T_c maintenance in BAT of cold-exposed rats through the inhibition of 5′-deiodinase and consequent reduction of T_3 concentrations that are critical for BAT function (González Pondal et al., 1995; Paier et al., 1997). Physiological changes associated with increased BAT thermogenesis during acute or chronic cold exposure include increased BAT temperature, UCP-1 protein synthesis, and sensitivity to NE injection (Doi and Kuroshima, 1982; Flaim et al., 1976; Ricquier et al., 1979). NE administration induced ~340% increase in metabolism in mice acclimated to 4 °C versus 25 °C (Doi and Kuroshima, 1982). Thus, the response to chemicals that induce changes in NE turnover rates will be significantly altered in cold-acclimatized animals. Cold acclimatization also induces increases in the BMR (~3 to ~30%), causes left ventricular hypertrophy, and has been associated with tachycardia and hypertension. Unfortunately, alterations in BAT function following acute or chronic cold exposure have traditionally been ignored or unrecognized with respect to their potential effects on chemical toxicity profiles.

Of course, NST is an important mechanism of rewarming in animals that undergo bouts of hibernation and arousal during exposure to severely cold environments. During hibernation, T_c, blood flow, heart rate and oxygen consumption (metabolic rate) are dramatically decreased in a precisely controlled manner (Barnes, 1989). As hibernation occurs, there is a pattern of suppression of carbohydrate metabolism to a reliance on FFA metabolism, which corresponds with increased UCP-2 and UCP-3 expression in the white adipose tissue and skeletal muscle, respectively (Boyer et al., 1998; Carey et al., 2003). Hibernation-arousal cycles are associated with rapid increases in T_c and respiration that induce increases in ROS generation; the ability of hibernators to tolerate high levels of oxidative stress during arousal has stimulated research into the potential benefits of the hibernation phenotype for tolerance to chemical toxicity. Many of the early studies compared toxicant-induced responses in hibernators at extremely low T_c (4–10 °C) to those elicited in nonhibernators or animals that had aroused from hibernation (T_c=32–40 °C). Perhaps not surprisingly, hibernators were generally found to tolerate larger doses of a compound or experience morbidity/mortality at a slower overall rate (for review, see Fuhrman, 1946). However, hibernation does not provide protection against all drugs, as the toxicity of caffeine, strychnine and epinephrine was exacerbated ~10-fold in hibernators compared to active ground squirrels (Pfeiffer et al., 1939). More recently, the hibernation phenotype, in the absence of hypothermia, has shown protection against ischemia-reperfusion injury of the intestine suggesting that the molecular mechanisms

providing protection against states of low tissue oxygen delivery are not dependent on the presence of a low T_c (Kurtz et al., 2006). Hopefully, research into the genetic components that correlate with protection from stress in the hibernation phenotype will aid in the development of treatment strategies to mitigate toxicity responses in nonhibernators, such as man.

Core temperature responses to drug toxicity

Hypothermia

The predominant thermoregulatory response observed in rodents following chemical exposure is hypothermia. The depth and duration of the hypothermic response is directly related to the dose of the toxicant and the ambient temperature to which the animal is exposed. Ethanol represents one of the most widely recognized drugs to induce hypothermia, with a high incidence of accidental hypothermic deaths reported in humans during the cold winter months. It has been suggested that heat stress potentiates the toxicity of chemical compounds by eliminating the hypothermic effect that drug toxicity induces during exposure to cool environments (Gordon and Stead, 1986; Gordon et al., 1990). To directly test this hypothesis, Dyer and Howell (1982) examined the latency of the visual evoked response (VER) in rats treated with triethyltin (TET) at environmental temperatures that either supported (22 °C) or prevented (30 °C) hypothermia development. During 7-hour exposure to 30 °C hypothermia was prevented and the latency of the VER was significantly increased, suggesting a protective effect of hypothermia on TET toxicity (Dyer and Howell, 1982). Similarly, increased toxicity to sodium selenite was observed during brief exposure (~4 h) to warm temperatures that are expected to prevent hypothermia development (unfortunately, T_c was not measured in this study; Watanabe et al., 1990). Malcolm and Alkana (1983) showed a direct relationship between temperature and ethanol lethality in mice with the LD_{50} (dose that induces 50% mortality) for i.p. injected ethanol increasing by 64% as T_a was increased from 20 to 35 °C. Ethanol intoxication was associated with a dose-related hypothermia in mice exposed to 30, 25 and 20 °C. Proposed mechanisms to account for the beneficial effect of hypothermia on ethanol sensitivity include a decrease in ethanol-induced perturbation of brain membranes (Chin and Goldstein, 1981; Harris and Schroeder, 1981), a decrease in metabolic energy expenditure (Gearhart et al., 1993; Jaeger and Gearhart, 1982; Wang and Peter, 1975) and alterations of ethanol elimination rates (Finn et al., 1989; Romm and Collins, 1987). Hypothermia has been shown to induce reductions, rather than increases in ethanol elimination rates with a resultant increase in blood concentrations; thus, alteration in ethanol pharmacokinetics cannot explain the mechanisms by which hypothermia decreases sensitivity to this chemical (Finn et al., 1989; Romm and Collins, 1987).

As described by Bejanian et al. (1990), ethanol causes the organism to behave like a poikilotherm as T_c can be easily manipulated by T_a during intoxication. However, other studies indicate that ethanol-induced hypothermia results from a regulated decrease in the thermal setpoint as autonomic and behavioral effector mechanisms have been shown to support its development. In rats, oral ethanol administration caused the selection of cooler T_a in a thermal gradient compared to that observed in saline-treated controls, which corresponded to a 1 °C decrease in colonic temperature (Gordon et al., 1988). Similar results were obtained in mice, in which the i.p. injection of ethanol caused decreases in metabolic rate and the selection of cooler T_a in a thermal gradient, showing that both autonomic and behavioral thermoeffectors were evoked in defense of hypothermia development (Gordon and Stead, 1986). It is likely that the regulated nature of ethanol-induced hypothermia is dependent on a variety of factors, including dose, route of administration, and environmental temperature.

The protective effect of hypothermia against air pollutants is thought to reside in the depressive effect of reduced T_c on ventilation rate. Previous work has shown that mice are particularly sensitive to irritating properties of airborne materials and will alter their ventilation in a dose-dependent manner (Alarie, 1966). Slade et al. (1997) showed that mouse strain differences in ozone incorporation into the lung tissue were directly related to the magnitude and duration of hypothermia. For example, when C3H/HeJ (C3) and C57BL/6J (C6) mice were inhalationally exposed to ozone, it was observed that C3 mice were more resistant to the toxic effects of this chemical than C6 mice. Post-hoc analysis of the T_c data indicated that the resistant C3 strain developed hypothermia at a greater rate and spent a longer amount of time in hypothermia than the more sensitive C6 strain, which was thought to be responsible for the differences in ozone toxicity. Although ventilation rate was not directly tested in these strains, it is reasonable to assume that the lower T_c level achieved in the C3 mice was associated with reduced ventilation rates and less ozone delivery to the lungs (Slade et al., 1997). Gearhart et al. (1993) hypothesized that the beneficial effects of hypothermia on chloroform-exposed mice were related to effects on metabolism, the partitioning of chemicals between the blood and tissues, and the rate of pulmonary ventilation, with depression of all of these responses occurring with a reduction in T_c. Using a physiologically based pharmacokinetic model (PBPK) to determine the effect of temperature on drug metabolism, mouse liver preparations were exposed to a wide range of temperatures and the Q_{10} rates of cytochrome P450 enzyme activities was found to be 2.2; the extrapolation of these data to the whole mouse suggested that for each 10 °C decrease in T_c, there was a >50% reduction in metabolic rate in chloroform-exposed mice (Gearhart et al., 1993). Similar results have been reported for mice exposed to ozone in which a ~10 °C decrease in T_c correlated with 60–80% reduction in minute volume (Bruce et al., 1979). These results were confirmed using an isolated lung tissue preparation in which reductions in T_c had a direct effect on the absorption of ozone across the respiratory epithelium (Postlethwaith et al., 1994). Taken together, these data indicate that reductions in T_c can influence multiple physiological mechanisms of inhalational toxicity.

The incidence of toxicant-induced hypothermia in humans in not as widely recognized as that in rodents (with the exception of ethanol intoxication), but has been documented in several

clinical cases following poisoning with OP pesticides (Cupp et al., 1975; Hassan et al., 1981; Klemmer et al., 1978). AChE inhibition levels as high as ~95% correlated with T_c reductions of ~5 °C (T_c=34 °C) in a woman accidentally poisoned with the OP insecticide diazinon (Hassan et al., 1981). In rats, serum AChE activity had to be reduced to only ~46% of control levels before animals became hypothermic following diisopropyl fluorophosphate (DFP) exposure (Gordon and Fogelson, 1993). The housing of rats in a relatively cool T_a of 22 °C following DFP poisoning facilitated hypothermia development, which explains the effectiveness of this compound following relatively mild inhibition of AChE (Gordon and Fogelson, 1993). The only clinical case documenting a beneficial effect of induced hypothermia was reported by Craig et al. (1959b) involving an 18-year-old man presenting with carbon monoxide poisoning. Carbon monoxide has a 200-fold greater affinity for hemoglobin than oxygen and induces hypoxia at the tissue level that accounts for the clinical symptoms associated with poisoning. Five hours following clinical admission his condition began to worsen as he developed fever (103.6 °F), severe hypotension (55/0) and a loss of bilateral reflexes. Ice packs were immediately placed around the patient and T_c reduced to 32 °C — following maintenance at this low T_c for ~32 h, the patient recovered full functional capabilities and was rewarmed and discharged with no abnormalities 2 weeks later (Craig et al., 1959a). Importantly, chlorpromazine was administered to prevent shivering as forced hypothermia (in the absence of a setpoint change) would have stimulated shivering and other reflexive mechanisms to increases T_c (Fig. 2C); such a stimulated increase in metabolic rate would have overridden the protective effects of reduced T_c. The protective effects of hypothermia in this case were likely due to reduced oxygen demands of the tissues and reduced production of reactive oxygen species (ROS) following the depression of metabolic rate with cooling. Hypothermia has shown protection for several clinical conditions associated with low tissue oxygen delivery, including cardiopulmonary bypass surgery, cerebral ischemia, and stroke (Dietrich and Kuluz, 2003; Marion et al., 1997).

Is OP-induced hypothermia due to dysfunction of the thermoregulatory system, or is it a regulated response mediated by a decrease in the thermal setpoint? There are several data to support the latter hypothesis, although the mechanisms by which this occurs in the POAH have not been clearly delineated. It was demonstrated as early as the 1960s by Meeter (1969) that increased tail skin blood flow is an important avenue of heat loss for the development of hypothermia in OP-poisoned rats. Following i.v. injection of soman, rats displayed a rapid increase in tail skin temperature that preceded a drop in T_c to ~32 °C — when the tail was wrapped in pre-heated cotton wool to reduce heat loss through this organ the rate of hypothermia development was reduced, indicating that this was an important organ for heat exchange (Meeter, 1969). To determine the regulated nature of this response, the rats were externally heated with a 60 W light bulb and reflexive adjustments in tail skin blood flow were recorded — once T_c was raised from 32 to ~33 °C, rat tail skin temperature increased abruptly as a reflexive mechanism to return it to its previous hypothermic level (Meeter, 1969). These studies are similar to those conducted by Liebermeister (see previous section on thermoregulatory control) on fever and suggest that OP-induced hypothermia represents a regulated reduction in response to a decrease in the thermal setpoint. More recently, Gordon (1997) showed that within 4 h of oral ingestion of chlorpyrifos, rats developed a profound reduction in T_c to 34.5 °C, which was behaviorally defended by the selection of cool T_a in a thermal gradient (Gordon, 1997). Chlorpyrifos-induced hypothermia is thought to be mediated by stimulation of cholinergic muscarinic receptors that activate heat loss pathways in the CNS. Anticholinergic agents, such as scopolamine have been used to examine mechanisms of OP-induced thermoregulatory changes in rodents. Scopolamine is the 6,7-epoxide of atropine that is more effective than atropine in eliciting actions within the CNS due to its ability to cross the blood-brain barrier. Gordon and Grantham (1999) showed a partial inhibitory effect of i.p. injected scopolamine on chlorpyrifos-induced hypothermia in rats — that is, hypothermia was reduced by ~50% immediately following scopolamine treatment, which occurred concomitantly with a robust increase in motor activity. Unfortunately, scopolamine treatment alone caused increases in T_c and motor activity that were difficult to dissociate from its specific effects on OP-induced mechanisms of hypothermia. However, to determine if scopolamine was mediating its actions at peripheral or central muscarinic receptors, rats were treated with methyl scopolamine, which is unable to penetrate the BBB and acts at peripheral muscarinic receptors only, and hypothermia was unaffected by this treatment. These data indicate that chlorpyrifos-induced hypothermia is mediated by cholinergic stimulation of heat loss pathways in the CNS thermoregulatory control centers and suggest that peripheral pathways play a minor role in this response (Gordon and Grantham, 1990).

Fever

Fever is defined as an elevation in T_c that occurs following a regulated increase in the thermal setpoint (see Fig. 2B). The beneficial effects of fever are related to more rapid resolution of inflammation and infection as immune responses are more effective at elevated T_c. For example, increased synthesis of proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α is important for the production of fever and acute phase proteins that aid in resolution of infections and inflammation (Horn et al., 1994; Kluger, 1991). There are several lines of evidence indicating that cytokines induce an increase in the thermal setpoint that results in fever (for a review, see Kluger, 1991). Interestingly, fever is a common response to chemical toxicity, which is presumably a response to tissue injury following subcutaneous, oral or inhalational exposures (Fine et al., 1997; Leftwich et al., 1982; Saadeh et al., 1996; Wood et al., 1983). In human volunteers, exposure to zinc oxide fumes caused a mild fever (~1 °C above baseline, termed "metal fume fever") that occurred with symptoms of cough, fatigue and myalgia (Fine et al., 1997). Symptoms associated with fever included chills and sweating, suggesting that this was

a true fever, rather than an unregulated hyperthermic response to inhalation of the chemical. Furthermore, IL-6 levels were significantly elevated at 6 h post-exposure, which corresponded with fever development (Fine et al., 1997). Zinc solutions have also been shown to induce IL-6 and TNF-α release in vitro from human peripheral blood monocytes (PBMCs) suggesting that these cells are a source for cytokine production (Falus and Beres, 1996). However, others have failed to detect increased circulating zinc levels following exposure to oxide fumes suggesting that inhaled zinc is not absorbed at high enough quantities to stimulate cytokine release from PBMCs (Blanc et al., 1991). It is more likely that the inhalation of zinc oxide fumes stimulates IL-6 release from alveolar macrophages, T or B lymphocytes as the lung receives direct exposure to the metal fumes (Fine et al., 1997).

In some cases of poisoning, excessive atropinization has been hypothesized as a mechanism for fever development. This is based on the concept that atropine can penetrate the BBB and antagonize CNS heat loss pathways that may be mediated by muscarinic receptors during fever development. Although incidence rates as high as 59% have been reported for atropinized patients presenting with fever within the first few hours after clinical presentation, ~25% of these patients maintained fever for several days following the discontinuation of atropine therapy (Christoph, 1989). Thus, alternate mechanisms appear to be mediating OP-induced fever.

In rodents, chlorpyrifos poisoning induces a biphasic response that consists of an initial hypothermia that lasts <24 h followed by a fever-like response that persists ≈72 h (Gordon et al., 1997). Does the fever-like response represent a rebound hyperthermia that occurs following hypothermic rewarming or is it a regulated response elicited by autonomic and behavioral effector responses that increase heat gain and decrease heat loss to drive T_c to an elevated level? There are several lines of evidence indicating that toxicant-induced fever is a regulated response due to an elevation in the thermal setpoint. First, if rats are exposed to a T_a of 31 °C during the first 24 h following chlorpyrifos dosing, hypothermia is prevented, but fever persists; thus, fever is independent of hypothermia in OP-poisoned rats (Gordon, 1997). Second, rats treated with sodium salicylate display attenuated fever following chlorpyrifos poisoning (Gordon et al., 1997). Sodium salicylate (aspirin) is an antipyretic (i.e., fever reducing) drug that blocks the activity of cyclooxygenase, an enzyme that converts arachidonic acid to prostaglandin E_2 (PGE_2) and other prostanoids. Increased PGE_2 synthesis in the POAH is thought to be responsible for the suppression of heat loss mechanisms and stimulation of heat producing/gain mechanisms that produce fever (Kluger, 1991). The effectiveness of sodium salicylate in attenuating chlorpyrifos-induced fever in rats supports the hypothesis that OPs induce fever through an increase in the thermal setpoint, which is mediated by increases in PGE_2 production (although PG levels were not measured in this study; Gordon et al., 1997). Third, chlorpyrifos-induced fever is behaviorally defended by rats in a thermal gradient by the selection of warm T_a that supports the elevation in T_c (Gordon, 1997). Based on these studies and the fever-like symptoms reported by patients, the elevation in T_c following chemical poisoning appears to be a regulated response. The question remaining is: what is the protective value of fever for recovery from chemical poisoning? Future studies are required to answer this question.

Perspectives

There are a variety of environmental conditions that can impact the thermoregulatory, cardiovascular, endocrinological and metabolic responses of homeotherms to chemical toxicity. The majority of studies used to determine the impact of thermal stress on chemical toxicity have been performed in small laboratory animals, such as frogs, fish, mice and rats. The advantage of these types of studies is primarily related to the ability of these species to withstand exposure to a wide range of environmental and chemical extremes that are unethical to study in human populations. Unfortunately, the extrapolation of research results from studies conducted in laboratory animals to the human condition has been hindered by a lack of understanding of the physiological differences that exist between these species. Heat stress is known to have a profound impact on chemical toxicity profiles as it has typically been associated with increased morbidity and mortality rates in several species. Although a detrimental effect of heat *per se* on chemical absorption profiles has been demonstrated *in vitro*, it has been suggested that it is the inhibition of hypothermia that is responsible for enhanced morbidity/mortality associated with the combination of heat and chemical exposure in the whole animal.

Hypothermia is the most common thermoregulatory response observed in small poikilotherms and homeotherms exposed to chemical toxicants, such as OP compounds and ethanol — although mild hypothermia has been observed in poisoned humans, the characteristics of the response differ significantly from that observed in rodents. Typically, the rate of hypothermia development is more rapid in small animals, as they are able to more effectively dissipate heat to the environment due to their large SA:M_b; additionally, the magnitude and duration of hypothermia is more pronounced in these species. How do these hypothermic characteristics alter the mechanisms of chemical toxicity? More importantly, how does one extrapolate findings from small species that readily develop hypothermia to a larger species, such as man for whom risk assessment guidelines are needed? Given the direct relationship between temperature and toxicity and the reduced incidence of hypothermia in man, it is likely that the predicted toxicity of a chemical for man will be *underestimated* from data provided from rodents in which hypothermia is commonly displayed during toxicant exposure (Gordon, 1991). As shown in many studies, hypothermia reduces metabolism and rate of toxicity such that survival times are significantly prolonged. Thus, the clinical use of induced hypothermia could impart a benefit by extending the window of opportunity to implement additional clinical intervention strategies to reverse toxicity and prevent long-term injury or death. The ability of clinically induced hypothermia to protect man against insults associated with low tissue oxygen availability (e.g., carbon monoxide poisoning) suggests that much can be learned by understanding the mechanisms of hypothermia

development in rodents and how this alters chemical toxicity profiles.

To understand the effect(s) that interactions between thermal stress and toxicant exposure have on physiological functioning of the whole organism, multiple factors must be taken into consideration. As described in this review, reflexive adjustments such as increases in ventilation rate, sweating and skin blood flow that occur in response to heat exposure can significantly increase chemical absorption and toxicity profiles. Unfortunately, there are additional factors, such as exercise (Rowsey et al., 2003. McMaster and Carney, 1986; McMaster and Finger, 1989), gender (Gordon and Mack, 2003; Gordon et al., 1997) and the time-of-day of exposure (Gordon and Mack, 2001) which can alter toxicity, but are generally not considered in experimental designs or the establishment of PELs. How do we most effectively replicate in a laboratory setting the multitude of factors that affect toxicity so that more appropriate safety exposure guidelines can be established for the variety of real-life scenarios that are encountered by human populations? Unfortunately, there is no straightforward answer to this question; however, if increased global warming is realized in the near future, the development of research models that more accurately simulate heat stress–toxicant exposures in humans will become increasingly important.

Conflict of interest disclosure statement

The author declares no conflicts of interest.

Acknowledgments

I thank Dr. L. Roszell for the opportunity to present these data at the Toxicology and Risk Assessment Conference in Cincinnati, OH (March, 2007). I also thank Dr. C.J. Gordon for his encouragement and invaluable insight into this field of study.

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In conducting the research described in this report, the investigators adhered to the “Guide for Care and Use of Laboratory Animals" as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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